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Nucleolytic Function of the Human DNA Repair Protein XRCC1

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XRCC1 is required for the repair of single-strand breaks resulting from DNA base damage, but the precise function of the protein is unknown. Recent studies have demonstrated that the absence of XRCC1 protein during mouse embryogenesis is lethal, and moreover that the protein directly interacts with Polymerase β and DNA ligase III, and also perhaps with poly(ADP-ribose) polymerase, proteins that act in the base excision repair pathway[1]. To determine the function of XRCC1, we expressed the protein from a bacterial vector that adds short peptide affinity tags to both termini. Fusion of the 15aa Ribonuclease-S peptide to the N-terminus of XRCC1 resulted in a significant increase in the level of soluble protein expressed. The combination of this fusion partner and a C-terminal polyhistidine tag facilitated affinity capture of the protein, and gel filtration was used to purify the full length protein away from truncation products. The purified protein forms an oligomer of 300 kDa, indicating a tetrameric conformation in solution, a feature shared by the p53 protein that has 3'->5' exonuclease activity[2]. In the presence of Mg^{2+}, XRCC1 catalyzed a limited release of nucleotide from untreated or nicked duplex DNA, and gave rise to heterogeneous size fragments from linearized plasmid DNA. To prove this nucleolytic activity is an intrinsic feature of the XRCC1 protein, we used an activity assay in a polyacrylamide gel following denaturing electrophoresis. As an additional indicator of function, the XRCC1 amino acid sequence was found to share significant similarity with the cell cycle proteins Dpb11 (*S. cerevisiae*), rad4/cut5 (*S. pombe*), and also p53 (human). Based on these sequence homologies and biochemical characterization, we propose a critical function for the XRCC1 nuclease in a regulatory or accessory role to polymerase β that acts in the final stage of the base excision repair pathway and that is essential for maintaining the integrity of the genome. (Work was done under the auspices of the U.S. DOE by LLNL under contract No. W-7405-ENG-48.)

1 Caldecott et al, Nucleic Acids Res. *in press*, 1996

2 Mummenbrauer et al, Cell 85, 1089-99, 1996